Human Exposures to Mutagens—An Analysis Using the Genetic Activity Profile Database

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The Genetic Activity Profile (GAP) database was used to identify and compare agents showing genotoxic activity in humans. The database revealed several substances for which both human and rodent cytogenetic data existed. Based on the ratio of the lowest effective doses (LEDs) in rodent versus human studies, humans appear to be at least 10 times more sensitive than rodents to the majority of the genotoxic substances examined. Several caveats are discussed which may be responsible, in part, for the apparent differences in sensitivity. Some of these differences could be due to variations in the test protocols or they may, in fact, reflect real differences between human and rodent cells. However, in contrast to the *in vivo* comparison, the LEDs for human data from *in vitro* studies were not uniformly lower than for comparable studies in rodents. The *in vitro* comparison suggests that the apparent differences in human versus rodent cell sensitivity seen *in vivo* must be viewed with a degree of caution. Nevertheless, the overall GAPs for these agents, and particularly the human *in vivo* data, underscore the concern for adequate protection of humans exposed to these environmental mutagens. — Environ Health Perspect 104(Suppl 3):585–589 (1996)

Key words: chemical potency, cytogenetics, database, human, rodent

For more than 10 years, the U.S. Environmental Protection Agency (U.S. EPA) and the International Agency for Research on Cancer (IARC) have collaborated in the development of an international database on the genetic and related effects of presumptive carcinogens to which humans are exposed. The U.S. EPA/IARC Genetic Activity Profile (GAP) database is a stand-alone personal computer software package for presentation of genetic toxicology data in both graphic and text formats. This database is available from the authors (M.D.Waters) and provides quantitative dose and effect information that is useful in guiding the selection of human biomonitoring techniques for field applications and laboratory-based investigations on agents of concern.

A comprehensive discussion of the GAP methodology is presented in Waters et al. (*1,2*). Briefly, graphic activity profiles are bar graphs with lines representing tests and identified by three-letter codes that are organized along the x-axis in either a phylogenetic or end point sequence (Figure 1). Values plotted on the y-axis are a logarithmic transformation of the lowest effective doses (LED) or highest ineffective doses (HID) reported in the literature. Positive test results extend vertically above the baseline to the mean LED while negative test results extend below the baseline to the mean HID. Dashed vertical lines represent a minority call for conflicting studies, and the lines extend to the extreme LED or HID. All data are original quantitative results abstracted from the published literature. The current database contains short-term test results on 565 agents evaluated by IARC and U.S. EPA, the latter including priority chemicals found at Superfund waste sites, pesticides, and hazardous air pollutants. A complete data record in GAP provides the chemical name, Chemical Abstracts Service registry number, a test code, test end point, qualitative test result, HID or LED, reference number, and a short citation. An example GAP based on phylogeny is presented in Figure 2 for ethylene oxide, an IARC Group 1 carcinogen (*3*) that has been demonstrated to induce genetic damage in occupationally exposed humans.

In constructing a GAP, doses derived from human studies are converted to milligrams per kilogram body weight (bw) per day of exposure using standard inhalation rates, body surface area, and body weight values for each sex. Certain assumptions were made because of the physical properties of compounds and routes of exposure (e.g., from inhalation of particles). Variation occurs in particle size of specific compounds and the rate of deposition in different areas of the respiratory system; however, 100% absorption was generally assumed. When reported, the dose at the target site was used. For example, in a study on lead exposure, the dose extracted was the measured blood level concentration in micrograms per milliliter. Similarly, doses obtained from *in vivo* mammalian tests were converted to milligram per kilogram bw per day of exposure, assuming 100% absorption and using standard weight and intake values for each sex and species of rodent, as reported by Gold et al. (*4*). For example, in a test using

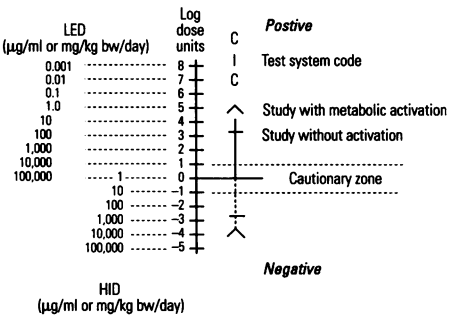

Figure 1. A schematic diagram of a genetic activity profile showing four studies for the test ECW (2 positive and 2 negative). The mean log dose unit of the majority call is indicated by a solid vertical bar. A dashed vertical bar indicates conflicting test results among the studies. Note, in cases where the number of positive and negative studies are the same, as illustrated here, the call is assigned positive.

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Abbreviations used: GAP, genetic activity profile; LED, lowest effective dose; U.S. EPA, U.S. Environmental Protection Agency; IARC, International Agency for Research on Cancer; HID, highest ineffective dose.

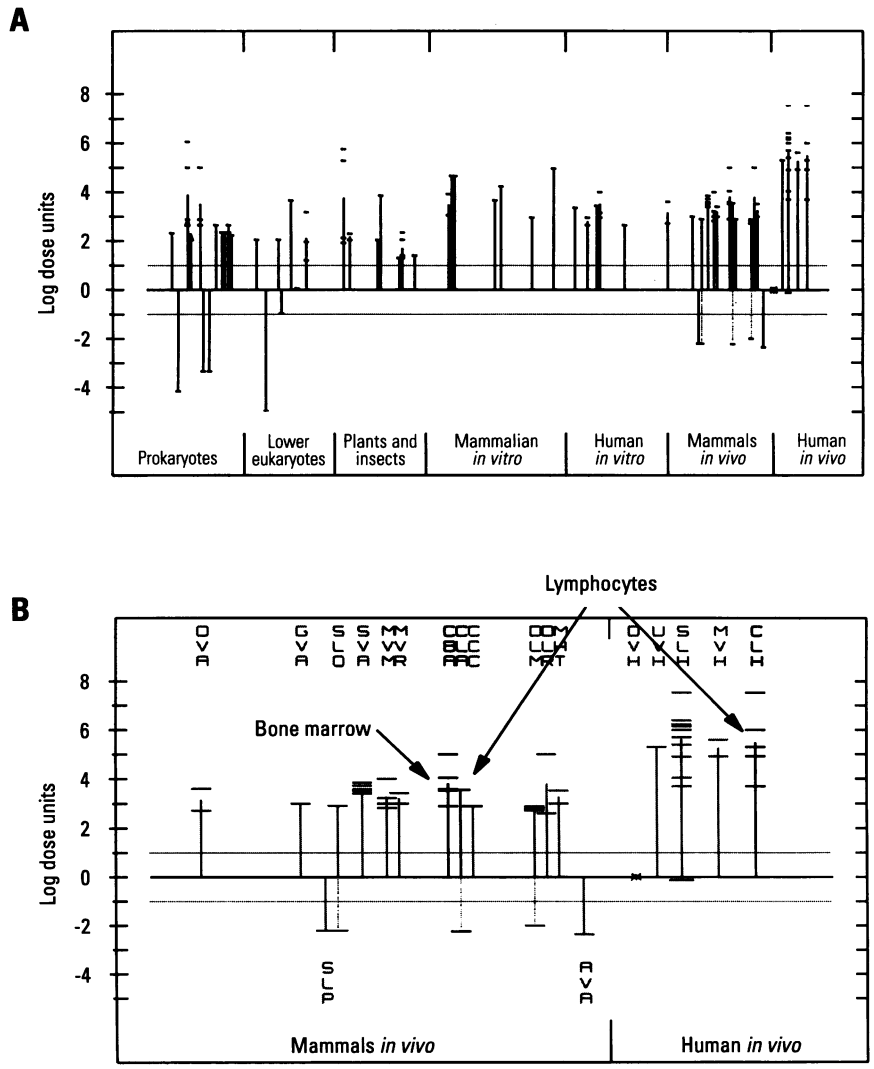

Figure 2. *A* is the genetic activity profile of ethylene oxide with tests organized phylogenetically. *B* shows the details of the *in vivo* mammalian tests for ethylene oxide. Bone marrow (CBA) and lymphocyte (CLA and CLH) tests are highlighted.

male mice fed 50 ppm of an agent in the diet, the standard food intake per day is 12% of the body weight, and the conversion is dose = 50 ppm × 12% = 6 mg/kg bw/day.

The multitest information in the GAP database has proved useful for the comparative assessment of both qualitative and quantitative results across several dimensions [e.g., concordance across species and end points (*1*), test battery selection (*5*), evaluation of relative potencies of agents (*6*), assessment of chemical structure–activity relationships (*7*), and evaluation of the activity of mixtures of chemicals (*8*)]. Here, the GAP database was used to identify and compare agents showing genotoxic activity in humans exposed to environmental mutagens.

The GAP database was searched to identify data resulting from *in vivo* human exposures and 41 substances were found (Table 1). Thirty-four of these substances had been evaluated for their ability to induce chromosomal damage in peripheral blood lymphocytes sampled from exposed individuals, while data on the remaining seven substances involved an assessment of other genotoxic end points, primarily sister chromatid exchanges. The mean LEDs or HIDs for chromosomal aberrations in human lymphocytes are provided in Figure 3 for the 34 substances, identified by exposure type and ordered by the magnitude of the exposure. Among the 21 substances that induced chromosomal damage, 12 involved occupational and/or environmental exposures and 9 involved medical treatments. Phosphine was the most potent substance in terms of dose (indicated by the height of the bar) while paracetamol was the least active in inducing genotoxic damage in humans.

It was of interest to compare the relative potency of these agents in humans to rodent systems. An analysis of the database resulted in 15 substances (8 occupational/environmental and 7 medical) for which both human and rodent cytogenetic data existed. Five animal studies involved the use of blood lymphocytes while 14 studies involved bone marrow cells (Figure 4). Based on the ratio of LEDs in rodent versus human studies, humans appear to be at least 10 times more sensitive than rodents to the majority of the genotoxic substances examined. Several caveats are involved in this comparison that may be responsible, in part, for the apparent difference in sensitivity. These include, for example, differences in the route of exposure (inhalation or oral in humans, intraperitoneal or oral in rodents) and in the type of exposure (predominantly chronic for humans, acute for animals).

To determine whether this differential sensitivity is also found for these substances when tested *in vitro*, seven substances were identified with both human and animal cell clastogenicity data (Figure 5). Again, the human data were limited to blood lymphocytes while the animal data were limited to transformed cells in culture, predominantly Chinese hamster ovary and Chinese hamster lung cell lines. In contrast to the *in vivo* comparison, the LEDs for human data were not uniformly lower than for rodents. Using a 10-fold difference in mean LED as an indicator of a significant difference in sensitivity, rodent cells appeared to be more sensitive to mercuric compounds while human cells appeared to be more sensitive to epichlorohydrin and myleran. Human and rodent cells appeared to be equally sensitive to thiotepa, bleomycin, and cyclophosphamide. Some of the apparent differences in sensitivity could be due to differences in cell type or protocol design or they may in fact reflect an intrinsic difference between human and rodent cells. The *in vitro* comparison suggests that the apparent differences in human versus rodent cell sensitivity seen *in vivo* (Figure 4) must be viewed with a degree of caution. However, the overall GAPs for these agents, and particularly the human *in vivo* data, should cause increased concern for

Table 1. Matrix of test results[a] for 41 compounds in the U.S. EPA/IARC GAP database.

Agents	CASRN	Exposure	SLH	SVH	CBH	CLH	AVH
Acrylonitrile	107-13-1	Occup/Envir				–	
Adriamycin	23214-92-8	Therapy	+			–	
Azathioprine	446-86-6	Therapy	–		?	+	
Benzene	71-43-2	Occup/Envir	–			+	
Bleomycin	11056-06-7	Therapy				+	
Caffeine	58-08-2	Food				–	
Chloroethylcyclohexylnitrosourea	13010-47-4	Therapy	+				
Chlorambucil	305-03-3	Therapy	+	+			
Chloroprene	126-99-8	Occup/Envir				+	
Cisplatin	15663-27-1	Therapy	+				
Cyclamate, sodium	139-05-9	Food				–	
Cyclophosphamide	50-18-0	Therapy	+			+	
Cyclosporin A	59865-13-3	Therapy				+	
Dichlorophenoxyacetic acid	94-75-7	Occup/Envir				–	
Dacarbazine	4342-03-4	Therapy		–			
1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane	50-29-3	Occup/Envir				+	
Epichlorohydrin	106-89-8	Occup/Envir				+	
Ethylene oxide	75-21-8	Occup/Envir	+			+	
5-Fluorouracil	51-21-8	Therapy	–			–	
Formaldehyde	50-00-0	Occup/Envir	+			+	
Isoniazide	54-85-3	Therapy				–	
Lead	7439-92-1	Occup/Envir	+			+	
Melphalan	148-82-3	Therapy	+			+	
6-Mercaptopurine	50-44-2	Therapy	+			+	
Mercuric compounds	7439-97-6	Occup/Envir	+			+	+
Methotrexate	59-05-2	Therapy	?		+		
Metronidazole	443-48-1	Therapy			–	–	
Myleran	55-98-1	Therapy	+			?	
Nickel	7440-02-0	Occup/Envir	+			+	
Paracetamol	103-90-2	Therapy				+	
Pentachlorophenol	87-86-5	Occup/Envir	–			+	
Phenylbutazone	50-33-9	Therapy			–		
Phenytoin	57-41-0	Therapy	+		–	–	
Phosphine	7803-51-2	Occup/Envir				+	
Styrene	100-42-5	Occup/Envir	–			–	
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1746-01-6	Occup/Envir				–	
Tetrachloroethylene	127-18-4	Occup/Envir	–			–	
Thiotepa	52-24-4	Therapy				+	
Toluene	108-88-3	Occup/Envir	–			–	
Vinyl chloride	75-01-4	Occup/Envir	+			+	
Xylenes	1330-20-7	Occup/Envir	–				

Abbreviations: CASRN, Chemical Abstracts Service Registry number; AVH, aneuploidy in human cells *in vivo*; CBH, chromosomal aberrations in human bone marrow cells *in vivo;* CLH, chromosomal aberrations in human lymphocytes *in vivo;* SLH, sister chromatid exchange in human lymphocytes *in vivo;* SVH, sister chromatid exchange in human cells *in vivo;* Occup/Envir, occupational/environmental. [a]Test results: +, positive; –, negative; ?, conflicting (1 positive and 1 negative).

adequate protection in humans exposed to these substances.

As demonstrated by this brief report, the current GAP database contains data on a wide range of end points used to assess genotoxic damage in humans exposed to occupational and environmental agents. While it contains information on several end points (e.g., sister chromatid exchange and DNA and protein binding) that are useful in monitoring human exposure, the GAP database does not contain information on newer methods such as ^{32}P-postlabeling for DNA adducts and the use of the single cell gel or comet assay to monitor for DNA single-strand breaks or alkali-labile damage. In view of the large number of studies conducted using these techniques, it is anticipated that the GAP database will be modified in the near future to incorporate this additional information.

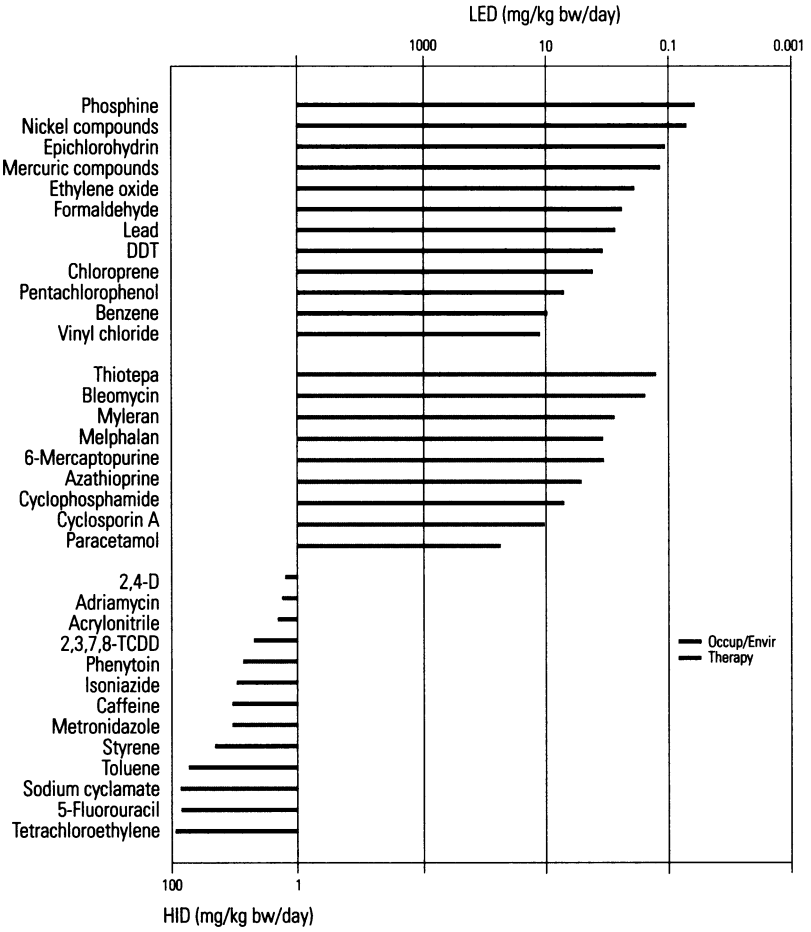

Figure 3. Rank order of agents based on mean doses (either the LED or HID) in the chromosomal aberration test following human exposure. Abbreviations: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; 2,4-D, dichlorophenoxyacetic acid; 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; Occup/Envir, occupational/environmental. The effective doses are grouped by the type of exposure, and the ineffective doses are mixed exposure types.

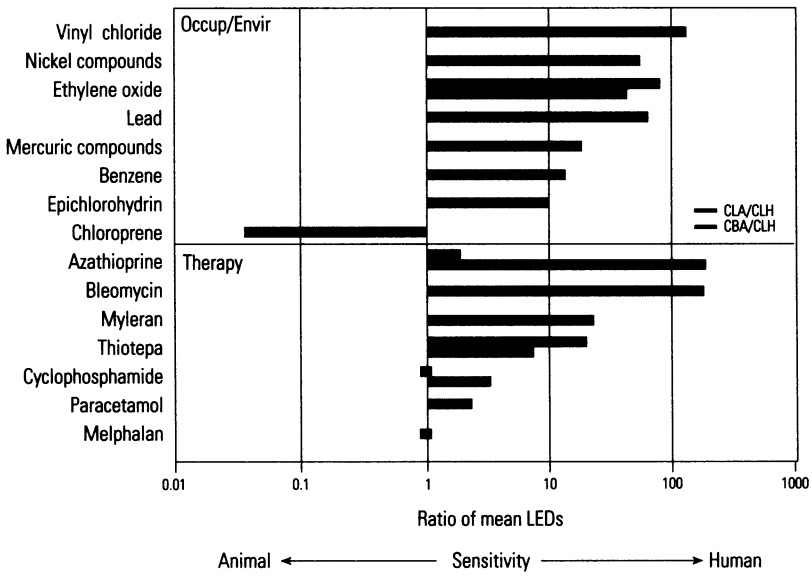

Figure 4. Ratio of mean LEDs from *in vivo* studies of chromosomal aberrations in animal bone marrow (CBA) and lymphocytes (CLA) relative to aberrations in human lymphocytes (CLH). Occup/Envir, occupational/environmental. A ratio greater than 1 indicates that the mean effective dose in human studies is a more sensitive indicator of genotoxicity for the chemical than the mean LED in the comparable animal studies. Conversely, values less than 1 indicate the animal tests are more sensitive than the human tests.

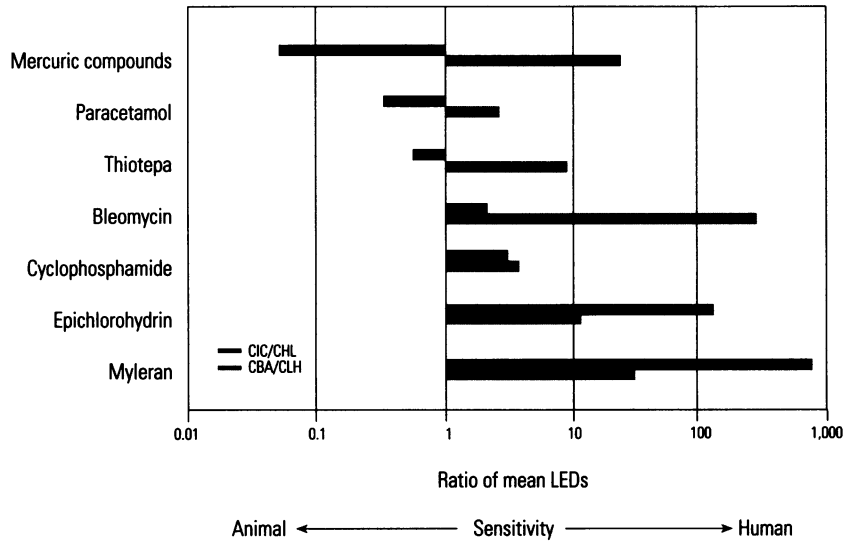

Figure 5. Ratios (CIC/CHL) of mean LEDs from *in vitro* studies of chromosomal aberrations in Chinese hamster cells (CIC) relative to aberrations in human lymphocytes (CHL). A ratio greater than 1 indicates that the mean effective dose in human cells is a more sensitive indicator of genotoxicity for the chemical than the mean LED in the comparable animal cells. *In vivo* data (CBA/CLH) from Figure 4 are repeated here for comparison.

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